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Instability of pathologic human extravillous trophoblast cells in culture. To define human extravillous trophoblast (HEVT) cells as a unique cell type among human cells in culture, we examined whether these cells exhibit differential susceptibility to the various types of cytotoxic stimuli and whether they exhibit the characteristics of those cells in vivo. To this end, we cultured the cells from the maternal side of the placenta and examined the cytotoxic susceptibility to granulocyte macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNF-alpha). By the transwell filter assay and 48-h cytotoxicity assay, the placental cells were susceptible to the cytotoxicity induced by both GM-CSF and TNF-alpha when compared with the nonplacental cells. In addition, we examined the surface markers of the

cells by using flow cytometry and determined the ability to fuse with the human cytotrophoblast cell line, JEG-3. The cells obtained from the placenta were initially cultured in "albumin medium," which contained 0.2% bovine serum albumin and 10% fetal bovine serum. The cells adapted to the albumin medium and expanded in culture for 12-15 passages. The cells were then transferred to the proliferation medium, which contains 0.2% bovine serum albumin, and 10% fetal bovine serum. At this point, cells cultured in albumin medium were transferred to the proliferation medium, and the cells were then harvested and maintained in the albumin medium. To examine the mycoplasma contamination, cells cultured in albumin medium, proliferation medium, and human fibroblast growth medium were examined by Southern blot analysis and the staining of mycoplasma antigens. To determine the cell fusion ability, all of these cells were cultured in the non-differentiation medium containing 0.2% bovine serum albumin and 0.25 mM IBMX. The cells transferred to the proliferation medium and the non-differentiation medium were examined by immunofluorescence staining and immunoe

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